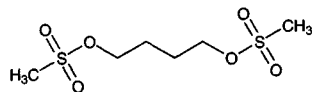


Busulfan



Molecular formula: C₆H₁₄O₆S₂

Molecular weight: 246.31

CAS Registry No.: 55-98-1

Merck Index: 1529

SAMPLE

Matrix: blood

Sample preparation: 500 μ L Plasma + 20 μ L 40 μ g/mL 1,6-bis(methanesulfonyloxy)hexane in ethyl acetate + 125 μ L 82 mg/mL diethyldithiocarbamate (DDTC) in water. Vortex. Add 200 μ L MeOH and 2 mL ethyl acetate. Vortex for 10 s and mix by rotation for 5 min. Centrifuge sample at 600 g for 10 min. Transfer organic phase to a glass tube, dry under nitrogen. Reconstitute extract with 100 μ L MeOH, vortex, inject a 30 μ L aliquot.

HPLC VARIABLES

Guard column: Nova-Pak ODS

Column: 150 \times 3.9 Nova-Pak ODS

Mobile phase: MeOH:water 80:20

Flow rate: 0.8

Injection volume: 30

Detector: UV 251; MS, electrospray, source temperature 120°, cone voltage 22-26 V

CHROMATOGRAM

Retention time: 8.5

Internal standard: 1,6-bis(methanesulfonyloxy)hexane (16)

Limit of detection: 20 ng/mL (S/N 6)

Limit of quantitation: 60 ng/mL

OTHER SUBSTANCES

Noninterfering: metabolites, allopurinol, carboplatin, cyclosporine, diazepam, heparin, lorazepam, methotrexate, methylprednisolone, ondansetron, pentoxifylline, phenytoin, prochlorperazine

KEY WORDS

derivatization; validation; plasma

REFERENCE

Heggie, J.R.; Wu, M.; Burns, R.B.; Ng, C.S.; Fung, H.C.; Knight, G.; Barnett, M.J.; Spinelli, J.J.; Embree, L. Validation of a high-performance liquid chromatographic assay method for pharmacokinetic evaluation of busulfan, *J. Chromatogr. B*, **1997**, 692, 437-444.

SAMPLE

Matrix: blood

Sample preparation: Condition a 1 mL Sep-Pak SPE cartridge with seven 1 mL portions of MeOH and two 1 mL portions of water. Add 20 μ L 40 μ g/mL CGA-112913 solution to 200 μ L plasma, vortex, add 200 μ L MeCN, vortex for 30 s, centrifuge at 16750 g for 3 min. Add 400 μ L water to a 360 μ L aliquot of the supernatant then add 200 μ L 1.17 M diethyldithiocarbamate in water, vortex for 30 s, rotate for 5 min, add 2 mL ethyl acetate, vortex for 1 min, centrifuge at 5125 g for 10 min. Evaporate a 1.8 mL aliquot of the ethyl acetate layer to dryness under a stream of air at 45°, reconstitute the residue in 200 μ L MeOH. Add 500 μ L water then the residue dissolved in MeOH to the SPE cartridge and allow them to pass through, wash with two 1 mL portions of MeOH:water 50:50, elute with two 250 μ L portions of MeOH and two 500 μ L portions of ethyl acetate, combine the eluates, evaporate to dryness under a stream of air at 45°, reconstitute the residue with 200 μ L mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 µm Microsorb-MV (Rainin)

Mobile phase: MeCN:THF:water 55:20:25 (pH 4.2 without modification)

Flow rate: 1.2

Injection volume: 100

Detector: UV 254

CHROMATOGRAM

Internal standard: CGA-112913 N-(2,6-difluorobenzoyl)-N-(3,5-dichloro-4-(3-chloro-5-trifluoromethylpyridin-2-yloxy)phenyl)urea

Limit of detection: 150 ng/mL

KEY WORDS

pharmacokinetics; plasma; derivatization; rat; SPE

REFERENCE

Chow,D.S.-L.; Bhagwatwar,H.P.; Phadungpojna,S.; Andersson,B.S. Stability-indicating high-performance liquid chromatographic assay of busulfan in aqueous and plasma samples, *J.Chromatogr.B*, **1997**, 704, 277–288.

SAMPLE

Matrix: blood

Sample preparation: 300 µL Plasma + 600 µL MeOH, mix, let stand at -20° for 20 min, centrifuge at 1500 g for 10 min. Add 600 µL supernatant to 150 µL 5% diethyldithiocarbamate and 600 µL 100 mM pH 5.5 ammonium acetate, mix, extract with 1.5 mL ethyl acetate, centrifuge for 1.5 min. Lyophilize a 1 mL aliquot of the extract, reconstitute with 200 µL MeOH, inject an aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 µm µBondapak C18

Mobile phase: MeOH:water 80:20

Flow rate: 1

Detector: UV 251

CHROMATOGRAM

Retention time: 12.0

Limit of detection: 200 nM

KEY WORDS

plasma; derivatization; pharmacokinetics

REFERENCE

Henner,W.D.; Furlong,E.A.; Flaherty,M.D.; Shea,T.C.; Peters,W.P. Measurement of busulfan in plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1987**, 416, 426–432.

SAMPLE

Matrix: blood

Sample preparation: Condition a 1 mL Bond Elut C18 SPE cartridge with 1 mL MeOH and 3 mL water. Add 1 mL plasma to the cartridge, wash with 2 mL water, dry, elute with 1 mL MeOH by centrifuging. Remove the eluate and add it to 1 mL 4 M NaI in water and 400 µL n-heptane, heat the mixture in a closed vial with stirring at 70° for 40 min, cool to room temperature. Remove 350 µL of the upper layer and centrifuge it at 12000 g. Remove 250 µL of the upper organic layer and add it to 100 µL 2-methoxy-ethanol, evaporate the n-heptane without heating under vacuum for 8 min, inject a 20 µL aliquot of the residue.

HPLC VARIABLES

Guard column: 50 × 4.6 5 µm LiChrosorb CN

Column: 250 × 4.6 5 µm LiChrosorb CN

Mobile phase: MeOH:water 20:80

Flow rate: 1

Injection volume: 20

Detector: UV 226 following photolysis with a GTE G8T5 germicidal lamp using a 0.8 mm i.d. PTFE knitted tube reactor, internal volume 2.4 mL (or a 25 m × 0.3 mm i.d. tube in a commercial Beam Booster reactor)

CHROMATOGRAM

Retention time: 16.5

Limit of detection: 20 ng/mL

KEY WORDS

plasma; post-column photolysis; derivatization; SPE; pharmacokinetics

REFERENCE

Blanz, J.; Rosenfeld, C.; Proksch, B.; Ehninger, G.; Zeller, K.-P. Quantitation of busulfan in plasma by high-performance liquid chromatography using postcolumn photolysis, *J. Chromatogr.*, **1990**, 532, 429–437.

SAMPLE

Matrix: blood

Sample preparation: Condition a 1 mL Bakerbond C18 SPE cartridge with three 1 mL portions of MeOH and two 1 mL portions of water, do not allow to dry. 300 µL Plasma + 30 µL 25 µg/mL IS in MeOH + 150 µL reagent solution, vortex for 10 s, add 2 mL ethyl acetate, vortex briefly, rock for 10 min on a blood mixer, centrifuge at 1500 g for 10 min. Remove 1 mL of the organic layer and evaporate it to dryness under a stream of air at 70°, reconstitute the residue in 500 µL MeOH, add 500 µL water, add to SPE cartridge, wash with two 1 mL portions of MeOH:water 50:50, elute with two 250 µL portions of MeOH, inject a 20 µL aliquot. (The reagent solution was 8.2 g sodium diethyldithiocarbamate in 100 mL water.)

HPLC VARIABLES

Column: 150 × 4 3 µm MicroPak-SP-C18

Mobile phase: MeCN:water:THF 55:25:20

Flow rate: 0.8

Injection volume: 20

Detector: UV 278

CHROMATOGRAM

Retention time: 4.5

Internal standard: N-(2,6-difluorobenzoyl)-N'-[3,5-dichloro-4-(3-chloro-5-trifluoromethyl-pyridin-2-yloxy)phenyl]urea (CGA-112913) (5.5)

Limit of detection: 0.4 ng/mL

KEY WORDS

plasma; SPE; derivatization

REFERENCE

MacKichan, J.J.; Bechtel, T.P. Quantitation of busulfan in plasma by high-performance liquid chromatography, *J. Chromatogr.*, **1990**, 532, 424–428.

SAMPLE

Matrix: blood

Sample preparation: 500 µL Serum + 100 µL water + 5 mL diethyl ether:dichloromethane 70:30, shake for 5 min, centrifuge at 1500 g for 5 min, freeze in dry ice/acetone. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, recon-

stitute the residue in 250 μ L water, inject a 100 μ L aliquot onto column A with mobile phase A ($t = 0$) for derivatization, after 5 min ($t = 5$) backflush the (derivatized) contents of column A onto column B with mobile phase B, after 3 min ($t = 8$) remove column A from the circuit and continue to elute column B with mobile phase B. After another 2 min ($t = 10$) direct the effluent from column B onto column C for 0.6 min ($t = 10.6$) then elute column C with mobile phase C, monitor the effluent from column C.

HPLC VARIABLES

Column: A $10 \times 4.5 \mu\text{m}$ Inertsil ODS-80A; B $150 \times 4.6 \mu\text{m}$ Inertsil ODS-2; C $150 \times 6.5 \mu\text{m}$ Capcell Pak C18 (Shiseido)

Mobile phase: A 1% sodium diethyldithiocarbamate (Use a $10 \times 4.5 \mu\text{m}$ Inertsil ODS-80A column between pump and injector to protect column A, replace every day. Stop flow of mobile phase A from $t = 5.1$ to $t = 32.$); B MeCN:20 mM pH 4.6 KH_2PO_4 60:40; B MeCN:20 mM pH 4.6 KH_2PO_4 65:35

Flow rate: A 0.5; B 1; C 1

Injection volume: 100

Detector: UV 278

CHROMATOGRAM

Retention time: 16

Limit of quantitation: 10 ng/mL

KEY WORDS

serum; column-switching; derivatization; heart-cut

REFERENCE

Funakoshi, K.-i.; Yamashita, K.; Chao, W.-f.; Yamaguchi, M.; Yashiki, T. High-performance liquid chromatographic determination of busulfan in human serum with on-line derivatization, column switching and ultraviolet absorbance detection, *J. Chromatogr. B*, **1994**, *660*, 200–204.

Butabarbital

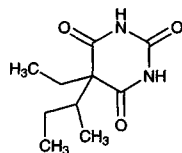
Molecular formula: $C_{10}H_{15}N_2NaO_3$

Molecular weight: 234.23

CAS Registry No.: 143-81-7, 125-40-6 (free acid)

Merck Index: 1530

Lednicer No.: 1 268



SAMPLE

Matrix: blood

Sample preparation: 200 μ L Serum + 200 μ L 50 μ g/mL hexobarbital in MeCN + 25 μ L glacial acetic acid, vortex for 10 s, centrifuge for 1 min, inject a 30-100 μ L aliquot of the supernatant.

HPLC VARIABLES

Column: μ Bondapak C18

Mobile phase: Gradient. MeCN:7.5 g/L NaH_2PO_4 adjusted to pH 3.2 with phosphoric acid 5:95 to 22:78 over 24 min, to 45:55 over 10 min, maintain at 45:55 for 5 min. Re-equilibrate with 5:95 for 5 min.

Column temperature: 50

Flow rate: 3

Injection volume: 30-100

Detector: UV 210

CHROMATOGRAM

Retention time: 15.0

Internal standard: hexobarbital (20.6)

Limit of detection: 200-2000 ng/mL

OTHER SUBSTANCES

Extracted: acetaminophen, amobarbital, butalbital, chlordiazepoxide, diazepam, ethchlorvynol, flurazepam, glutethimide, methaqualone, methypylon, nitrazepam, pentobarbital, phenobarbital, phenytoin, primidone, salicylic acid, secobarbital, theophylline

Simultaneous: amitriptyline, caffeine, clomipramine, codeine, desipramine, ethotoin, imipramine, lidocaine, mesantoin, methsuximide, nirvanol, nortriptyline, oxazepam, procainamide, phenylpropanolamine, propranolol, quinidine

KEY WORDS

serum

REFERENCE

Kabra,P.M.; Stafford,B.E.; Marton,L.J. Rapid method for screening toxic drugs in serum with liquid chromatography, *J.Anal.Toxicol.*, **1981**, 5, 177-182.

SAMPLE

Matrix: blood

Sample preparation: Prepare an SPE cartridge by plugging the end of a 1 mL disposable pipette tip with glass wool and adding about 100 mg Chromosorb P/NAW. Add 50 μ L plasma then 50 μ L 10 μ g/mL tolylphenobarbital in 200 mM HCl to the SPE cartridge, let stand for 2 min, elute with 1 mL chloroform:isopropanol 6:1. Evaporate the eluate to dryness under a stream of nitrogen at 30°, reconstitute the residue in 100 μ L mobile phase, inject a 15 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 4.6 5 μ m Supelcosil-LC-8

Mobile phase: MeCN:water 20:80

Flow rate: 3.3

Injection volume: 15

Detector: UV 208

CHROMATOGRAM

Retention time: 4.18

Internal standard: tolylphenobarbital (7.57)

Limit of detection: 50-100 ng/mL

OTHER SUBSTANCES

Extracted: theophylline, caffeine, barbital, ethosuximide, primidone, carbamazepinediol, phenacemide, methyprylon, nirvanol, phenobarbital, carbamazepine epoxide, mephentoin, pentobarbital, amobarbital, carbamazepine, glutethimide, phenytoin, secobarbital, methaqualone

Noninterfering: acetaminophen, amikacin, amitriptyline, clonazepam, cyclosporine, desipramine, diazepam, digoxin, disopyramide, gentamicin, imipramine, lidocaine, methotrexate, N-acetylprocainamide, netilmicin, nortriptyline, procainamide, quinidine, salicylic acid, sulfamethoxazole, tobramycin, trimethoprim, valproic acid, p-hydroxyphenobarbital, vancomycin

Interfering: chloramphenicol

KEY WORDS

plasma; SPE

REFERENCE

Svinarov,D.A.; Dotchev,D.C. Simultaneous liquid-chromatographic determination of some bronchodilators, anticonvulsants, chloramphenicol, and hypnotic agents, with Chromosorb P columns used for sample preparation, *Clin.Chem.*, **1989**, 35, 1615-1618.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4 OmniPac PAX-500 (Dionex)

Mobile phase: Gradient. A was MeCN:5 mM sodium carbonate 9:81. B was MeCN:20 mM sodium carbonate 20:80. A:B from 100:0 to 0:100 over 10 min.

Flow rate: 1

Detector: UV 254

CHROMATOGRAM

Retention time: 9

OTHER SUBSTANCES

Simultaneous: allobarbital, amobarbital, barbital, barbituric acid, mephobarbital, metha-barbital, methohexital, phenobarbital, phenytoin, secobarbital, thiamylal

REFERENCE

Slingsby,R.W.; Rey,M. Determination of pharmaceuticals by multi-phase chromatography: Combined reversed phase and ion exchange in one column, *J.Liq.Chromatogr.*, **1990**, 13, 107-134.

SAMPLE

Matrix: solutions

Sample preparation: Prepare a 0.5 mg/mL solution in MeOH, inject a 5 µL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 150 mM phosphoric acid and 50 mM triethylamine. B was MeCN:water 80:20 containing 150 mM phosphoric acid and 50 mM triethylamine. A:B 100:0 for 2.2 min then to 0:100 over 30 min.

Column temperature: 30

Flow rate: 2

Injection volume: 5

Detector: UV 210

CHROMATOGRAM

Retention time: 15.3

OTHER SUBSTANCES

Simultaneous: acetaminophen, aprobarbital, chlordiazepoxide, chloroxylenol, chlorpromazine, clenbuterol, cortisone, danazol, diflunisal, doxapram, estrone, fluoxymesterone, mefenamic acid, methyltestosterone, nicotine, oxazepam, phentermine, phenylpropanolamine, progesterone, sulfamethazine, sulfanilamide, testosterone, testosterone propionate, tranlycypromine, tripeleennamine

KEY WORDS

details for purification of triethylamine in paper

REFERENCE

Hill,D.W.; Kind,A.J. The effects of type B silica and triethylamine on the retention of drugs in silica based reverse phase high performance chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 3941–3964.

SAMPLE

Matrix: solutions

Sample preparation: Dissolve in mobile phase to a concentration of 50 µg/mL.

HPLC VARIABLES

Column: 250 × 4 β-cyclodextrin polymer-coated silica (Chromatographia 1993, 36, 373)

Mobile phase: MeOH:water 50:50

Flow rate: 0.6

Injection volume: 20

Detector: UV 240

CHROMATOGRAM

Retention time: k' 1.66

OTHER SUBSTANCES

Simultaneous: aprobarbital, pentobarbital, amobarbital, butalbital, secobarbital, thiopental, phenobarbital

REFERENCE

Forgács,E.; Cserhádi,T. Retention behaviour of barbituric acid derivatives on a β-cyclodextrin polymer-coated silicon column, *J.Chromatogr.A*, **1994**, *668*, 395–402.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30

Flow rate: 2

Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenpropfen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiaicol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarboxtyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephénytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyl dopa, methyl dopamine, methylphenidate, methylprednisolone, methyltestosterone, methypylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfasoxazole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranlycypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripeleminamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill, D.W.; Kind, A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J. Anal. Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

Sample preparation: Dissolve in mobile phase at a concentration of 100 µg/mL, inject a 5 µL aliquot.

HPLC VARIABLES

Column: 300 × 2 µm Bondapak C18

Mobile phase: MeCN:water 30:70 adjusted to pH 3.0 with formic acid

Flow rate: 0.27

Injection volume: 5

Detector: MS, VG TRIO 2000 single quadrupole MS with EI or CI or UV 270

CHROMATOGRAM

Retention time: 9.5

OTHER SUBSTANCES

Simultaneous: butethal, butalbital, talbutal, amobarbital, pentobarbital

KEY WORDS

mass spectra given

REFERENCE

Ryan, T.W. Identification of barbiturates using high performance liquid chromatography-particle beam EI/CI mass spectrometry, *J. Liq. Chromatogr.*, **1994**, *17*, 867-881.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 µm Supelcosil LC-DP (A) or 250 × 4 5 µm LiChrospher 100 RP-8 (B)

Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)

Flow rate: 0.6

Injection volume: 25

Detector: UV 229

CHROMATOGRAM

Retention time: 5.60 (A), 4.92 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyrindamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephentermine, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyl-dopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen,

nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfapyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS

also details of plasma extraction

REFERENCE

Koves, E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, 692, 103–119.

SAMPLE

Matrix: solutions

Sample preparation: Prepare a 1-10 µg/mL solution in water, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 µm Hypersil SCX/C18

Mobile phase: MeCN:25 mM pH 3 Na₂HPO₄ 50:50

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: k' 0.85

OTHER SUBSTANCES

Also analyzed: amitriptyline, barbital, benzoic acid, clomipramine, clonazepam, desipramine, diazepam, flurazepam, furosemide, imipramine, nitrazepam, phenobarbital, phenol, phenolphthalein, pindolol, propranolol, resorcinol, salicylic acid, secobarbital, terbutaline, xylazine

KEY WORDS

effect of mobile phase pH on capacity factor is discussed

REFERENCE

Walshe, M.; Kelly, M.T.; Smyth, M.R.; Ritchie, H. Retention studies on mixed-mode columns in high-performance liquid chromatography, *J.Chromatogr.A*, **1995**, 708, 31–40.

SAMPLE

Matrix: urine

Sample preparation: 2 mL Urine +1 mL 500 mM pH 5.5 phosphate buffer, add to an Extrelut 3 SPE cartridge, let stand for 10 min, elute with 15 mL dichloromethane:isopropanol 95:5. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 µL mobile phase, inject a 20 µL aliquot.

HPLC VARIABLES

Guard column: 4 × 4 5 µm Lichrospher 100 RP8

Column: 250 × 4 5 µm Lichrospher 100 RP8

Mobile phase: Gradient. MeCN:10 mM pH 4.4 phosphate buffer from 30:70 to 40:60 over 8 min, maintain at 40:60 for 6 min, to 30:70 over 1 min

Flow rate: 1

Injection volume: 20

Detector: UV 212

CHROMATOGRAM

Retention time: 8.7

Limit of detection: 300 ng/mL

OTHER SUBSTANCES

Extracted: barbital, allobarbital, phenobarbital, pentobarbital, secobarbital

Noninterfering: acetaminophen, aspirin, amitriptyline, buprenorphine, caffeine, carbamazepine, chlorpromazine, desipramine, dextromethorphan, doxepin, ephedrine, fenfluramine, imipramine, lidocaine, loxapine, meperidine, methadone, methaqualone, naloxone, naltrexone, nicotine, orphenadrine, oxycodone, papaverine, pentazocine, phendimetrazine, phenmetrazine, phentermine, phenylpropanolamine, phenytoin, primidone, procaine, promethazine, propoxyphene, propylphenazone, theobromine, theophylline, trazodone, triflupromazine, trimethoprim, trimipramine

KEY WORDS

SPE

REFERENCE

Ferrara, S.D.; Tedeschi, L.; Frison, G.; Castagna, F. Solid-phase extraction and HPLC-UV confirmation of drugs of abuse in urine, *J. Anal. Toxicol.*, **1992**, 16, 217-222.

Butacaine

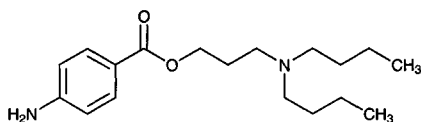
Molecular formula: $C_{18}H_{30}N_2O_2$

Molecular weight: 306.45

CAS Registry No.: 149-16-6, 149-15-5 (sulfate)

Merck Index: 1531

Lednicer No.: 1 12



SAMPLE

Matrix: solutions

Sample preparation: Prepare a 10 µg/mL solution in MeOH, inject a 20 µL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica

Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7

Flow rate: 2

Injection volume: 20

Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 2.0

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazepine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyrindamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamlamine, meclorphenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nifemesine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phenoltamine, phenylephrine, phenyltoloxamine, physostigmine, pimindone, pimezide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, pir tramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, pro-

thipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranlycypromine, trazodone, trifluoperazine, trifluoperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripeleppamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane, I.; McKinnon, A.; Flanagan, R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, 323, 191-225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30

Flow rate: 2

Detector: UV 210

OTHER SUBSTANCES

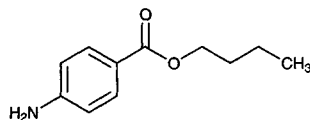
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepam, brompheniramine, buprenorphine, buspirone, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapson, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fentanyl, fencamfamine, fenpropafen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaicol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarboxystyrene, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephentyoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyl dopa, methyl dopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mi-bolone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, per-

santine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sulfadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfasoxazole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranlycypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripeleminamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

- Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233-242.

Butamben



Molecular formula: $C_{11}H_{15}NO_2$

Molecular weight: 193.25

CAS Registry No.: 94-25-7, 577-49-0 (picrate)

Merck Index: 1538

SAMPLE

Matrix: solutions

Sample preparation: Inject a 5 μ L aliquot.

HPLC VARIABLES

Column: 300 \times 4 10 μ m μ Bondapak C18

Mobile phase: MeCN:MeOH:water 20:20:60 containing 0.06% sulfuric acid, 0.5% sodium sulfate, and 0.02% sodium heptanesulfonate, pH 2.6

Flow rate: 2

Injection volume: 5

Detector: UV 305

CHROMATOGRAM

Retention time: 14

OTHER SUBSTANCES

Simultaneous: benzocaine, lidocaine, pramoxine, procaine, tetracaine

REFERENCE

Menon, G.N.; Norris, B.J. Simultaneous determination of tetracaine and its degradation product, p-n-butylaminobenzoic acid, by high-performance liquid chromatography, *J.Pharm.Sci.*, **1981**, 70, 569-570.

Butethal

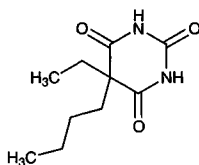
Molecular formula: $C_{10}H_{16}N_2O_3$

Molecular weight: 212.25

CAS Registry No.: 77-28-1

Merck Index: 1550

Lednicer No.: 1 268



SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μ L MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μ L aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 \times 4.6 5 μ m Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A: B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 200.5

CHROMATOGRAM

Retention time: 14.858

KEY WORDS

whole blood

REFERENCE

Gaillard, Y.; Pépin, G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J. Chromatogr. A*, **1997**, 763, 149-163.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 \times 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30

Flow rate: 2

Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepam, brompheniramine, buprenorphine, buspirone, butabarbital, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenpropfen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarboxtyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyl dopa, methyl dopamine, methylphenidate, methylprednisolone, methyltestosterone, methylpyrrolone, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nyldrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phenacyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfasoxazole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamine, tolazoline, tobutamide, tolmetin, tranlycypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill, D.W.; Kind, A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J. Anal. Toxicol.*, **1994**, *18*, 233-242.

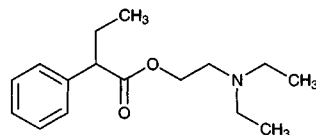
Butethamate

Molecular formula: C₁₆H₂₅NO₂

Molecular weight: 263.38

CAS Registry No.: 14007-64-8, 3639-12-1 (citrate)

Merck Index: 1551



SAMPLE

Matrix: solutions

Sample preparation: Prepare a 10 µg/mL solution in MeOH, inject a 20 µL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica

Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7

Flow rate: 2

Injection volume: 20

Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 2.5

OTHER SUBSTANCES

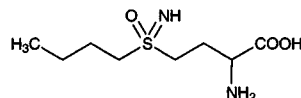
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzocetamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazepine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyrizidamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamlamine, meclorphenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypropazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, pen-thienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phen-tolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozone, pin-dolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piri-tramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolin-tane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, pro-thipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine,

quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocinide, tolpropamine, tolycaine, tranlycypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripeleennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane, I.; McKinnon, A.; Flanagan, R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191-225.

Buthionine sulfoximine



Molecular formula: C₈H₁₈N₂O₃S

Molecular weight: 222.31

CAS Registry No.: 5072-26-4

Merck Index: 1556

SAMPLE

Matrix: blood

Sample preparation: 100 μ L Plasma + 50 μ L 100 μ g/mL L-norvaline, add 10% sulfosalicylic acid, mix, centrifuge at 13000 rpm for 5 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 50 μ L 400 mM pH 9.53 phosphate buffer, add 50 μ L 3 mg/mL dansyl chloride in acetone, heat at 40° for 10 min, inject a 5 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 4.6 Zorbax ODS

Mobile phase: MeCN:MeOH:10 mM pH 2.1 phosphate buffer 3:1:8

Column temperature: 40

Flow rate: 1

Injection volume: 5

Detector: F ex 335 em 525

CHROMATOGRAM

Retention time: 5

Internal standard: norvaline (10)

Limit of detection: 10 μ g/mL

KEY WORDS

derivatization; rat; plasma; protect from light

REFERENCE

Koyama,H.; Sugioka,N.; Hirata,I.; Ohta,T.; Kishimoto,H. Determination of L-buthionin (SR)-sulfoximine, γ -glutamylcysteine synthetase inhibitor in rat plasma with HPLC after prelabeling with dansyl chloride, *J.Chromatogr.Sci.*, **1996**, 34, 326-329.

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. 200 μ L Plasma + 200 μ L 100 μ g/mL L-norleucine in 100 mM HCl, vortex, filter (Millipore 10000 nominal molecular weight limit) while centrifuging at 5000 g for 15 min. Remove a 40 μ L aliquot of the ultrafiltrate and add it to 80 μ L freshly-prepared EtOH:phenylisothiocyanate:triethylamine 40:1:1, vortex, let stand at room temperature for 15 min, add 10 μ L 316 mg/mL L-serine, let stand for 15 min, evaporate to dryness under reduced pressure, reconstitute with 600 μ L A, inject a 250 μ L aliquot. Urine. Condition a Bond Elut C18 SPE cartridge with 3 mL MeOH and 3 mL water. 100 μ L Urine + 100 μ L 100 μ g/mL L-norleucine in 100 mM HCl, vortex, add to the SPE cartridge, elute with 300 μ L 100 mM HCl, elute with 500 μ L MeCN:100 mM HCl 30:70, collect all the effluent from the cartridge, vortex thoroughly. Remove a 40 μ L aliquot and add it to 80 μ L freshly-prepared EtOH:phenylisothiocyanate:triethylamine 40:1:1, vortex, let stand at room temperature for 15 min, add 10 μ L 316 mg/mL L-serine, let stand for 15 min, evaporate to dryness under reduced pressure, reconstitute with 600 μ L A, inject a 250 μ L aliquot.

HPLC VARIABLES

Guard column: 10 \times 4.6 5 μ m Adsorbosphere c18

Column: 250 × 4.6 5 µm Adsorbosphere C18

Mobile phase: Gradient. A:B 91.5:8.5 for 30 min, to 55:45 over 10 min, to 0:100 over 2 min, maintain at 0:100 for 6 min, return to initial conditions over 2 min, re-equilibrate for 8 min. A was MeCN:140 mM pH 6.40 sodium acetate buffer containing 500 µL/L triethylamine and 200 µL/L 1 mg/mL EDTA 6:94. B was MeCN:water 60:40 containing 200 µL/L 1 mg/mL EDTA.

Flow rate: 1.25

Injection volume: 250

Detector: UV 254

CHROMATOGRAM

Retention time: 26.0 (S), 26.6 (R)

Internal standard: L-norleucine (39.3)

Limit of detection: 1 µg/mL (plasma)

Limit of quantitation: 2 µg/mL (plasma), 10 µg/mL (urine), 6 µg/mL (urine)

KEY WORDS

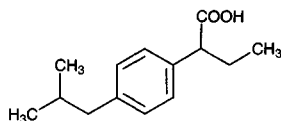
derivatization; plasma; ultrafiltrate; SPE

REFERENCE

Brennan, J.M.; O'Dwyer, P.J.; Ozols, R.F.; LaCreta, F.P. High-performance liquid chromatographic determination of the *S*- and *R*-diastereoisomers of L-buthionine (*SR*)-sulfoximine in human plasma and urine, *J.Chromatogr.*, **1993**, 620, 121–128.

Butibufen

Molecular formula: $C_{14}H_{20}O_2$
Molecular weight: 220.31
CAS Registry No.: 55837-18-8
Merck Index: 1557



SAMPLE

Matrix: bulk, formulations

Sample preparation: Raw materials. Dissolve in MeCN to a concentration of 10 mg/mL, inject a 3 μ L aliquot. Microemulsions. Dilute 0.5-2 g microemulsion to 5 mL with MeCN, add this to a previously activated C18 Sep-Pak SPE cartridge, wash four times with MeCN, collect eluate and make up to 10 mL with MeCN, inject a 3 μ L aliquot. Cream. 0.5-2 g Cream + 3 mL MeCN, shake vigorously at 55° for 10 min, add 3 mL MeCN, cool, centrifuge at 3500 rpm for 20 min. Remove the supernatant and filter (0.2 μ m), repeat extraction three times, make up to 25 mL with MeCN, pass through a previously conditioned Sep-Pak C18 cartridge, inject a 15 μ L aliquot. Microencapsulated formulations. Weigh out an amount of microencapsulated formulation corresponding to 50-300 mg butibufen, dilute with 2 mL water, stir at 40° until the coating had dissolved, make up to 10 mL with MeCN, filter (0.45 μ m), inject a 3 μ L aliquot. Tablets, sachets. Pulverize, weigh out amount containing 250-1500 mg butibufen, add 10 mL water, stir at 40° for 15 min, dilute to 50 mL with MeCN, filter (0.45 μ m), inject a 3 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 3.9 4 μ m Novapak C18

Mobile phase: MeCN:water:orthophosphoric acid 472:548:0.4

Column temperature: 40

Flow rate: 1

Injection volume: 3-15

Detector: UV 264

CHROMATOGRAM

Retention time: 10.1

KEY WORDS

SPE; microemulsions; creams; microencapsulated; tablets; sachets

REFERENCE

González Tavares,L.; Pérez de la Cruz,M.J.; Sanz Saiz,P.; Camacho,M.A.; Martin,J.L. High pressure liquid chromatographic determination of the new non-steroidal anti-inflammatory agent butibufen, *Arzneimittelforschung*, **1992**, 42, 818-820.

Butorphanol

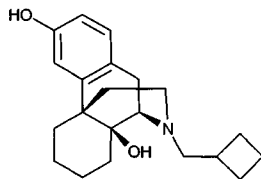
Molecular formula: C₂₁H₂₉NO₂

Molecular weight: 327.47

CAS Registry No.: 42408-82-2, 58786-99-5 (tartrate)

Merck Index: 1565

Lednicer No.: 2 325



SAMPLE

Matrix: blood

Sample preparation: Condition a Sep-Pak C18 SPE cartridge with water, MeOH, and 100 mM ammonium acetate. Add 200 µL plasma to the SPE cartridge, wash with 100 mM ammonium acetate, elute with MeOH:100 mM ammonium acetate 3:1. Evaporate the eluate to dryness under reduced pressure, dissolve the residue in 200 µL mobile phase, inject a 20 µL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 Hitachi gel 3056 octadecylsilica

Mobile phase: MeOH:100 mM ammonium acetate 60:40

Flow rate: 1

Injection volume: 20

Detector: MS, Hitachi M1000, APCI, nebulizer 260°, vaporizer 399

CHROMATOGRAM

Retention time: 3.8

Limit of detection: 0.5-2.5 ng/mL

OTHER SUBSTANCES

Simultaneous: atipamezole, atropine, flumazenil, ketamine, medetomidine, midazolam, xylazine

KEY WORDS

plasma; SPE; dog

REFERENCE

Kanazawa,H.; Nagata,Y.; Matsushima,Y.; Takai,N.; Uchiyama,H.; Nishimura,R.; Takeuchi,A. Liquid chromatography-mass spectrometry for the determination of medetomidine and other anaesthetics in plasma, *J.Chromatogr.*, **1993**, 631, 215-220.

SAMPLE

Matrix: solutions

Sample preparation: Dissolve in mobile phase.

HPLC VARIABLES

Guard column: 15 × 3.2 7 µm Applied Biosystems pre-column

Column: 100 × 2 10 µm µPorasil

Mobile phase: MeCN:5 mM pH 3.75 sodium acetate 80:20

Flow rate: 1

Injection volume: 200

Detector: UV 214

CHROMATOGRAM

Retention time: 9.31

Limit of detection: 6.3 ng/mL

OTHER SUBSTANCES

Simultaneous: buprenorphine, morphine, ethylmorphine, codeine, nalbuphine, nalorphine, meperidine, tramadol

Noninterfering: thiopentone, succinylcholine, pancuronium, diazepam, atropine, neostigmine

Interfering: fentanyl

REFERENCE

Ho,S.-T.; Wang,J.-J.; Ho,W.; Hu,O.Y.-P. Determination of buprenorphine by high-performance liquid chromatography with fluorescence detection: application to human and rabbit pharmacokinetic studies, *J.Chromatogr.*, **1991**, 570, 339–350.

SAMPLE

Matrix: urine

Sample preparation: Condition a 1 mL Cyano Bond Elut SPE cartridge with 2 mL MeOH then 2 mL 10 mM pH 6.0 ammonium acetate, do not allow to dry. 1 mL Urine + 100 μ L 1 M pH 6.0 ammonium acetate + 50 μ L 500 ng/mL IS in water, vortex for 20 s, add to SPE cartridge, wash with 2 mL 10 mM pH 6.0 ammonium acetate, wash with 2 mL MeCN, dry cartridge under vacuum for 1 min. Elute with two 1 mL portions of MeCN: triethylamine 99:1. Evaporate eluate to dryness under nitrogen at 30°, reconstitute in 150 μ L MeCN:MeOH:water 20:10:70 containing 10 mM ammonium acetate and 10 mM tetramethylammonium hydroxide adjusted to pH 5.0 with glacial acetic acid, vortex for 30 s, inject a 75 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m octyl (Jones Chromatography)

Mobile phase: MeCN:MeOH:water:buffer 20:10:69:1 (Buffer was 1 M ammonium acetate and 1 M tetramethylammonium hydroxide adjusted to pH 6.0 with glacial acetic acid.)

Flow rate: 1

Injection volume: 75

Detector: F ex 200 em 325

CHROMATOGRAM

Retention time: 23.4

Internal standard: BC-2605 (cyclopropyl analog of butorphanol) (13.0)

Limit of quantitation: 1 ng/mL

OTHER SUBSTANCES

Simultaneous: acetaminophen, antipyrine, procainamide, aspirin, lidocaine, furosemide, dextrorphan, dextromethorphan, metabolites

Noninterfering: cimetidine, ibuprofen, N-acetylprocainamide

KEY WORDS

SPE

REFERENCE

Wiley,T.A.; Duncan,G.F.; Tay,L.K.; Pittman,K.A.; Farmen,R.H. High-performance liquid chromatographic method for the quantitative determination of butorphanol, hydroxybutorphanol, and norbutorphanol in human urine using fluorescence detection, *J.Chromatogr.B*, **1994**, 652, 171–178.